PREPARE Study

(PREemptive Pharmacogenomic testing for Preventing Adverse drug Reactions)

Statistical Analysis Plan

On behalf of The Ubiquitous Pharmacogenomics Consortium

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Change History:

Version	Date	Change	Original	Revised	Page in relevant version
1.0	**July 2017**	-	--	-	-
2.0	**March 2020**	Adjustment of Statistical Advisor	Dr. Erik van Zwet	Dr. Stefan Böhringer	1
			All analyses will be performed with standard statistical software package SAS and SPSS.	All analyses will be performed with standard statistical software package R and SPSS.	4
		Logistical changes as per amendments 6	See protocol version 5 and 6 change history	See protocol version 5 and 6 change history	-
		Accounting for time dependent variables Accounting for time dependent effects		Updates of DPWG guidelines: As per the study protocol, updates of DPWG guideline will be implemented throughout the study. If updates for certain drug/gene interactions result in no actionable therapeutic recommendation for all phenotypes, patients enrolled on this index drug will be removed from the intention to treat analysis. If updates result in a different recommendation, but is still actionable, patients remain in the analysis and a status-variable indicating recommendation change will be included as a covariate in the primary analysis.	17
				The association between the primary endpoint and time dependent variables such as date of enrolment, DPWG adherence rate and others will be investigated. When they are indeed associated, relevant variables will be included as a covariates in the primary analysis.	18

1. Introduction

This Statistical Analysis Plan (SAP) provides a detailed overview of the pre-planned analyses of the PREPARE study (PREemptive Pharmacogenomic testing for Preventing Adverse drug Reactions). This document does not include the analysis plan for the cost-effectiveness analysis, the follow-up analysis of extreme-phenotypes or the analysis of the drug-drug-gene sub-study.

The PREPARE study is performed in accordance with the World Medical Association Declaration of Helsink (1964) and the Tokyo (1975), Venice (1983), Hong Kong (1989) and South Africa (1996) amendments and will be conducted in compliance with the protocol, Standard Operating Procedures (SOPs) and the ICH- GCP.

These planned analyses will be performed by the PhD Candidate under the supervision of the statistical advisor. The results of these analyses will be described in a statistical analysis report, to be used as the basis of the primary research publications. All analyses will be performed with standard statistical software package R and SPSS. The finalised analysis datasets, programs and outputs will be archived following ICH-GCP guidelines.

2. Definitions

ADE	Adverse drug event
ADR	Adverse drug reaction
CONSORT	Consolidated Standards of Reporting Trials
DPWG	Dutch Pharmacogenomics Working Group
Index drug	One of 39 drugs for which a patient is included in the study (see Table 1)
eCRF	Electronic Case Report Form
IQT	Inter-quartile range
PGx	Pharmacogenomics
SD	Standard deviation
Serious ADR	An ADR resulting in hospitalization or prolongation or hospitalization or death
Subsequent drug	The drug of interest (see Table 1) which a patient initiates during follow-up. Initiation of one of these drugs initiates a similar follow-up as for the index drug

3. Study design and objectives

This is multi-center, open, randomized, cluster cross-over implementation study conducted in seven countries across Europe. Countries will be block randomised to start with either PGx-guided prescribing (study arm) or standard of care (control arm) for a period of 19 months (and an additional 3 months follow-up). After this enrolment period, a new set of patients will be recruited and the opposite strategy will implemented for a period of 18 months (and an additional 3 months follow-up). All study patients will be followed-up for a minimum of 12 weeks; maximum follow-up is limited to 22 and 21 months per patient, corresponding to which time block they are enrolled.

The primary objective is to determine whether implementing pre-emptive PGx testing of an entire panel of clinically relevant PGx markers, to guide the dose and drug selection for over 39 commonly prescribed drugs, will result in an overall reduction in the number of clinically relevant drug-genotype associated adverse drug reactions (ADRs). We hypothesize that the implementation of PGx-guided drug prescribing will reduce both the occurrence and severity of drug-genotype associated ADRs in comparison to patients receiving standard of care treatment.

3.1. Sample size calculation

The sample size calculation is based on showing a significant reduction of clinically relevant drug-genotype associated ADRs among the subgroup of patients carrying an actionable drug-genotype combination.

The frequency of the composite endpoint grade 2,3,4,5 (defined as a “clinically relevant ADR”) is estimated to be 0.04. We however hypothesize that this frequency is higher among those with an aberrant genotype and could range between 0.04 and 0.10.

We hypothesize that implementing PGx-guided drug and dose selection among those carrying an actionable index drug-gene combination will lead at least a 30% reduction in occurrence of these clinically relevant drug-genotype associated ADRs. If the frequency of the grade 2,3,4,5 endpoint is 0.10 and the true frequency for the carriers of an actionable index drug-gene combination PGx-guided subjects is 0.0683 a sample size of 1,200 subjects in the study arm and 1,200 subjects in the control is still sufficient to reject the null hypothesis with a probability (power) 0.8 and a Type I error probability of 5%.

Preliminary data from a pilot experiment among 200 patients indicate approximately 30% of included patients will carry an actionable genotype for the index drug. Therefore, 4,000 subjects of unknown genotype in the study arm and 4,000 subjects of unknown genotype in the control arm are needed.

To compensate for a 1.25% dropout rate, 100 extra patients will be included in the study. This equals a total inclusion of 8,100 patients.

In this cluster cross-over study, it might be necessary to adjust the sample size for correlation within clusters (design effect). The design effect was estimated by a simulation. A treatment effect of 3.2% (lowering from 0.1 to 0.068) was assumed on average with a standard deviation of 1% for the treatment effect across clusters (95% of clusters are expected to show between 1.2% and 4.2% ADRs in treated subjects). Required sample size was very similar to the sample size calculation based on independent individuals implying that the design effect is close to 1. A design effect of 1 was assumed for the final sample size.

4. Interim analysis

No interim analyses are planned.

5. Study Outcomes Defined

5.1. Primary outcome

The primary outcome is the occurrence of at least one causal (definite, probable or possible), clinically relevant (classified as NCI-CTCAE grade 2, 3, 4, or 5), drug-genotype specific ADR, attributable to the index drug, within 12 weeks of follow-up. For oncology patients receiving 5-FU, capecitabine, tegafur or irinotecan, only hematological toxicities of NCI-CTCAE grade 4-5 and non-haematological toxicities of NCI-CTCAE grade 3-5 will be considered clinically relevant.

The primary outcome endpoint variable is binary and will be summarized using percentages.

All collected ADEs during the follow-up period will be assessed with regard to severity, causality and the association with genotype (Figure 1).

The severity of the ADE is classified using the CTCAE (version 4.0) classification scale (1) for the primary analysis and the WHO classification (serious/non-serious) for the secondary analysis. This assessment will be performed by the local research team. A random 10% sample of this assessment will be re-performed independently by Lareb. Lareb is blinded to the patients' arm allocation. If assessments are not in agreement, then a consensus will be made. If a consensus cannot be reached, then Lareb's assessment is directive. See 4.9 PREPARE SOP Adverse Drug Event Assessment of Severity for detailed information on how severity is assessed.

The causality assessment will be performed using the Liverpool Causality Assessment Tool (LCAT) (2). Once an ADE has been assessed regarding causality it is referred to as an ADR. This assessment will be performed by the local research team. A random 10% sample of this assessment will be will be re-performed independently by Lareb. Lareb is blinded to the patients' arm allocation. If assessments are not in agreement, then a consensus will be made. If a consensus cannot be reached, then Lareb's assessment takes precedence. See 4.8 PREPARE SOP Adverse Drug Event Assessment of Causality for detailed information on how causality is assessed.

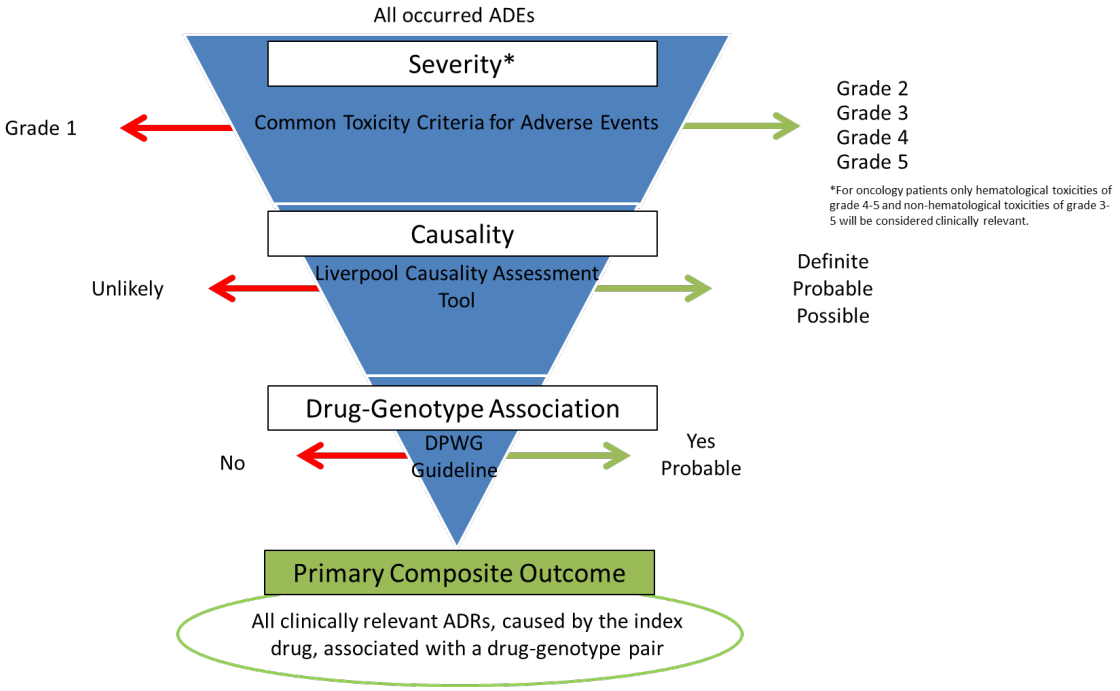

Figure 1 Funnel plot of classification of ADEs which contribute to the primary endpoint as drug-genotype specific ADRs

Finally, the association between the ADR, the index drug and the genotype is assessed using the DWPG guidelines (3, 4). This analysis will be performed in bulk by an expert panel when the study data collection is completed, to ensure all analyses are performed using the same version of the DPWG guidelines. This expert panel is blinded to the patients' arm allocation. See 4.10 PREPARE SOP Adverse Drug Event Assessment of Drug-Genotype Association for detailed information on how severity is assessed.

Only clinically relevant adverse drug events, categorized as definitely, probably or possibly caused by the drug (i.e. now constituting an ADR), which have a drug-genotype association will contribute to the primary endpoint, as highlighted in green in Figure 1.

5.2. Secondary outcomes

1. The primary analysis will be repeated by treating NCI-CTCAE grade (2, 3, 4, or 5) as ordinal outcome. Logistic ordinal regression will be used in the analysis.

2. Occurrence of at least one ADR which contributes to the primary composite endpoint within the entire follow-up of the study. This regards a clinically relevant ADR which was caused either by the index drug of inclusion or any subsequent drug. This endpoint variable is binary and will be summarized using percentages.
    a. Since the PGx intervention is a panel approach, the intervention may be of use in multiple prescriptions of multiple drugs in a patient's lifetime. This secondary

analysis will be performed to quantify the effect of PGx guided prescribing to guide drug and dose selection of multiple drugs, for the treatment of a single patient.

3. Occurrence of at least one serious ADR within 12 weeks of follow-up. This regards a clinically relevant ADR which was caused by the index drug of inclusion. This endpoint variable is binary and will be summarized using percentages.

4. Number of self-reported ADEs through the LIM survey, irrelevant of severity or drug-gene association, but attributed to the index drug, experienced by each patient during the entire study follow-up. This variable is numerical and will be summarized using the mean, standard deviation and range (if the variable appears normal), or median, IQR and range (if it appears skewed).

5. Number of serious self-reported ADEs through the LIM survey, irrelevant of severity or drug-gene association, but attributed to the index drug, experienced by each patient during the entire study follow-up. This variable is numerical and will be summarized using the mean, standard deviation and range (if the variable appears normal), or median, IQR and range (if it appears skewed).

6. Number of dose adjustments made to the index drug experienced by each patient, during the entire study follow-up. This variable is numerical and will be summarized using the mean, standard deviation and range (if the variable appears normal), or median, IQR and range (if it appears skewed).

7. Drug cessation of index drug due to an ADR during the entire study follow-up. This variable is binary and will be summarized using percentages.

8. Drug cessations of index drug due to lack of efficacy during the entire study follow-up. This variable is binary and will be summarized using percentages.

9. Number of additional drugs that are prescribed during follow-up to each patient. This variable is numerical and will be summarized using the mean, standard deviation and range (if the variable appears normal), or median, IQR and range (if it appears skewed).

10. Routine drug levels (only those that are collected routinely) as a proxy for exposure. This variable is continuous and will be summarized using the mean, standard deviation and range (if the variable appears normal), or median, IQR and range (if it appears skewed) and will be reported per drug.

11. Difference in patient-reported drug adherence score between baseline and 18 months. This variable is numerical and will be summarized using the mean, standard deviation and range (if the variable appears normal), or median, IQR and range (if it appears skewed).

6. Inclusion and exclusion criteria

6.1. Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. Subject must be ≥ 18 years old
2. Subject must receive a 1st prescription (meaning no known prescription for this drug in the preceding 12 months) for a drug included in Table 1, which is prescribed to them in routine care.
3. Subject is able and willing to take part and be followed-up for at least 12 weeks
4. Subject is able to donate blood or saliva
5. Subject has signed informed consent

Table 1 List of drugs eligible for inclusion (n=39)

Antiarrhythmic	Flecainide
	Propafenon
Analgesic	Codeine
	Tramadol
Anticancer	Capecitabine
	Fluorouracil
	Irinotecan
	Tamoxifen
	Tegafur
Anticoagulation	Acenocoumarol
	Clopidrogel
	Phenprocoumon
	Warfarin
Antidepressant	Citalopram
	Escitalopram
	Paroxetine
	Sertraline
	Venlafaxine
Antidepressant (TCA)	Amitriptyline
	Clomipramine
	Doxepine
	Imipramine
	Nortryptiline
Antiepileptic	Phenytoin
Antihypertensive	Metoprolol
Anti-infective	Efavirenz
	Flucloxacillin
	Voriconazole
Antipsychotic	Aripiprazole
	Clozapine
	Haloperidol
	Pimozide
	Zuclopenthixol

Cholesterol-lowering	Atorvastatin
	Simvastatin
Immunosuppressive	Azathioprine
	Mercaptopurine
	Tacrolimus
	Thioguanine
Psychostimulant	Atomoxetine

6.2. Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Previous (direct-to-consumer, or clinical) genetic testing for a gene important to the index drug
2. Pregnancy or lactating
3. Life expectancy estimated to be less than three months by treating clinical team
4. Duration of index drug total treatment length is planned to be less than seven consecutive days. A drug whose route of administration changes during the first seven days (e.g. intravenous to oral flucloxacillin) but whose total treatment duration is seven days or longer, is still eligible.
5. For inpatients: hospital admission is expected to be less than 72 hours (to facilitate acting upon the PGX results)
6. Unable to consent to the study
7. Unwilling to take part
8. Subject has no fixed address
9. Subject has no current general practitioner
10. Subject is, in the opinion of the Investigator, not suitable to participate in the study
11. Patient has existing impaired hepatic or renal function for which a lower dose or alternate drug selection is already part of current routine care. This would not apply to any drugs specifically given to manage liver/renal impairment/transplantation.
12. Estimated glomerular filtration rate (MDRD) of less than 15 ml/min per 1,73m^2 in a subject with a functioning graft
13. Patients with advanced liver failure (stage Child-Pugh C)

When 10% patients on any index drug have been included in the study, recruitment of new patients on that drug will no longer be permitted. In other words, the inclusion of patients with a first prescription for the index drug is limited to 5% of the control arm and 5% of the study arm . More specifically 2.5%,in the control arm and 2.5% in the study arm in the first time-block, and likewise in the second time-block, as shown in Figure 2.

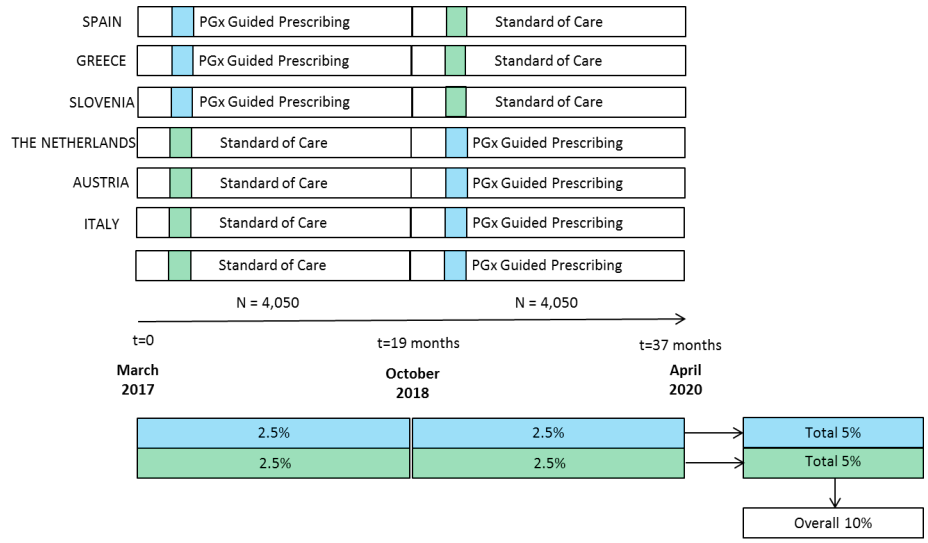

Figure 2 The number of patients included in the PREPARE study for a first prescription of an index drug is capped at 10% and will be monitored per arm.

7. Description of study population

7.1. Representativeness of study sample

Characteristics (age, sex, index drug and index drug indication) of all patients approached for the study will be recorded in a study log-book. These characteristics will be compared among those who have been approached to participate in the study and among those who consented to participate in the study. A CONSORT flow diagram will depict the representativeness of the sample and the sub-groups used for the primary analysis (see Appendix A).

7.2. Baseline comparability of standard of care and study arms

Baseline comparability of patients in the standard of care arm and the study arm will be performed among all patients included in the primary analysis. They will be summarized with respect to age, gender, country in which they were recruited, index drug for inclusion, actionability, global health score, number of co-medications, number of co-morbidities, number of drug allergies, BMI, ethnicity, exercise, alcohol consumption, smoking, education level and patient reported drug-adherence at baseline.

Categorical data will be summarised by numbers and percentages. Numerical data will be summarised by mean, SD and range if data appear normally distributed, or median, IQR and range if data appear skewed.

Tests of statistical significance will be undertaken for baseline characteristics to identify imbalance between the arms.

Table 2 Baseline comparison of standard of care arm with PGx guided prescribing arm

	Standard of care arm	PGx guided prescribing arm	p-value
N			
Age			
Gender			
Recruitment country **NL** **UK** **SLO** **GRE** **IT** **AUS** **ESP**			
Index drug for inclusion **Flecainide** **Propafenon** **Codeine** **Tramadol** **Capecitabine** **Fluorouracil** **Irinotecan** **Tamoxifen** **Tegafur** **Acenocoumarol** **Clopidrogel** **Phenprocoumon** **Warfarin** **Citalopram** **Escitalopram** **Paroxetine** **Sertraline** **Venlafaxine** **Amitriptyline** **Clomipramine** **Doxepine** **Imipramine** **Nortryptiline** **Phenytoin** **Metoprolol** **Efavirenz** **Flucloxacillin** **Voriconazole** **Aripiprazole** **Clozapine** **Haloperidol** **Pimozide** **Zuclopenthixol** **Atorvastatin** **Simvastatin** **Azathioprine** **Mercaptopurine** **Tacrolimus** **Thioguanine** **Atomoxetine**			
Actionable index drug-genotype **No actionable index drug-genotype**			
Global health score			
Number of co-medications			
Number of co-morbidities			
Number of drug allergies			
BMI			
Self -reported ethnicity **Caucasian** **Non-Caucasian**			

Exercise
Alcohol consumption
Education level **Completed doctorate** **Completed master's degree** **Completed bachelor's degree** **Completed high school** **Complete middle school** **Not (yet) completed middle school**
Patient reported drug adherence score

7.3. Completeness of follow-up

The number of patients who were lost to follow up within each treatment group will be reported in Appendix A and the reasons where known will be documented. Any deaths and their causes will be reported separately.

8. Follow-up assessments

The summary matrix for data collection is provided in Table 3. T=0 is defined as the day the patient initiates the index drug. During the 18-month follow-up period trained research nurses will contact patients at baseline (t=0) (± one week), four weeks (± two weeks) and 12 weeks (± three weeks) after inclusion, a cross-sectionally at the end of the time-block (± one month). If a visit is performed outside the windows defined, then this will be reported, but still used in the primary analysis.

Table 3 Summary of prospective data collection All in yellow is ONLY collected in the study arm

Variable Domains	Baseline	4 weeks	12 weeks	End of Study (end of time-blockcross-sectional)	2 Weeks	8 Weeks
PATIENTS						
Personal Characteristics						
1.1 General information	X					
1.2 In- and exclusion criteria	X					
1.3 Demographics	X					
1.4 Recruitment Information	X					
1.5 Health behaviours	X					
1.6 DNA sample collection	X					
1.7.1 PGx testing results	X					
1.7.2 Safety-code card	X					
1.8.1 Index drug prescription	X					
1.8.2 Action ability	X					
1.8.3 Index drug prescription change	X					
1.8.3.1 Contact information HCP	X					
1.8.3.1 Adherence to DPWG guidelines	X					
1.9 Control for logistics	X					
Clinical Monitoring						
3. Comorbidities and allergies	X	X	X	X		
4. Co-medication and herbal remedies	X	X	X	X		
Nurse Assessment : Clinical Outcome						
2.1 General information	X	X	X	X		
2.2 Index drug changes		X	X	X		
2.3 Drug adherence	X			X		
2.4 Global Health Score	X	X	X	X		
2.5 Quality of Life	X	X	X	X	X	X
2.6 Attitudes and knowledge	X			X	X	X
Adverse events						
5. Adverse drug events					X	X
5.1 Patient perception of ADE		X	X	X		
5.2 Healthcare costs associated with ADE		X	X	X		
5.3 Identifying the extreme phenotype		X	X	X		
5.4 ADE assessment of severity		X	X	X		
5.5 ADE assessment of causality		X	X	X		
5.6 ADE assessment of drug-genotype association		X	X	X		

9. Protocol compliance and monitoring

On-site monitoring will be undertaken by Catalyst Clinical Research. All sites were visited three times (after inclusion of approximately 10 patients, after inclusion of 50% of recruitment goal, and at site closure) by monitors from Catalyst who oversaw the progress of the study, ensured reported study data were accurate, complete and verifiable from source documents and that the study was conducted in compliance with the protocol, SOPs and ICH-GCP. 10% of all eCRFs and 100% of informed consent forms are checked for accuracy, SOP and protocol compliance.

10. Patient groups for analysis

Actionable index-drug gene interaction sub-population: the patients who have an actionable index drug-genotype interaction. This means that DPWG guidelines recommend a drug/dose change or additional clinical monitoring as a result of a specific phenotype.

No index-drug gene interaction sub-population: the patients who do not have an actionable index drug-genotype interaction. This means that the DPWG guidelines do not recommend a drug/dose change or additional clinical monitoring as a result of their PGx results.

All patients within the standard of care arm will be able to be divided into an actionable and a not actionable sub-population. See Appendix A.

All patients within the PGx guided prescribing arm will be able to be divided into an actionable and a not actionable sub-population. See Appendix A.11. Protocol deviations

Potential deviations of important protocol specifications are listed below. Any protocol deviations will be classified prior to data analysis. The number and percentage of patients with protocol deviations will be summarised by site and treatment group with details of type of deviation provided.

Protocol deviations:

- In PGx guided prescribing arm:
    - Patients whose HCPs receive their PGx information (for the gene of interest) more than one week after index drug initiation
- In standard of care arm:
    - Patients whose DNA sample arrives at the laboratory for analysis after more than one week
- In both arms:
    - Patients who do not use the index drug for a minimum of seven consecutive days

The intention-to-treat principle is the main strategy of the analysis adopted for the primary and secondary outcomes. Patients classified as having a protocol deviation will be included in the intention-to-treat analysis, but excluded in a per protocol analysis.

These analyses will be performed on all patients who provided data to at least one follow-up moment originating from the index drug (either at 4 weeks or at 12 weeks). Methods for handling missing data are discussed in section 14 'Analysis of missing data'.

Updates of DPWG guidelines:

As per the study protocol, updates of DPWG guideline will be implemented throughout the study. If updates for certain drug/gene interactions result in no actionable therapeutic recommendation for all phenotypes, patients enrolled on this index drug will be removed from the intention to treat analysis. If updates result in a different recommendation, but is still actionable, patients remain in the analysis and a status-variable indicating recommendation change will be included as a covariate in the primary analysis.

12. Analysis of primary outcome

Statistical comparison of the primary endpoint between the standard of care arm and PGx guided prescribing arm will be performed per country using logistic regression, since each country acts as its own control. A center-level covariate will be included as well as covariates representing any potentially confounding factors (age, number of drug allergies, number of comedications and global health score). Additionally, time-of-enrolment dependent variables such being treated with an updated of guidelines will be included as covariates depending on an independent model selection step (see below). These seven logistic regressions will be pooled in a meta-analysis using a forest plot.

The primary analysis is performed using a gatekeeping analysis:

1. A meta-analysis of the seven country specific logistic regressions will be performed to compare the percentage of patients reaching the primary endpoint, among the actionable sub-populations in the PGx guided prescribing arm and the control arm. See Figure 3, in red.

The pooled log odds of this analysis is representative of the impact of PGx guided prescribing on the primary endpoint.

Only when this is statistically significant a second analysis will be performed.

2. A meta-analysis of the seven country specific logistic regressions will be performed to compare the percentage of patients reaching the primary endpoint, among all patients included in the study in the PGx guided prescribing arm and the control arm. See Figure 3, in blue.

The pooled log odds of this analysis is representative of the impact of PGx guided prescribing on a population level.

In all analyses, standard strategies to ensure proper model fit will be employed (accounting for number of events, correlation between covariates, goodness-of-fit).

Accounting for time dependent effects:
The association between the primary endpoint and time-of-enrolment dependent variables such as date of enrolment itself, DPWG adherence rate at that time and others will be investigated for association with the primary endpoint without including arm. When they are indeed associated, relevant variables will be included as a covariates in the primary analysis.

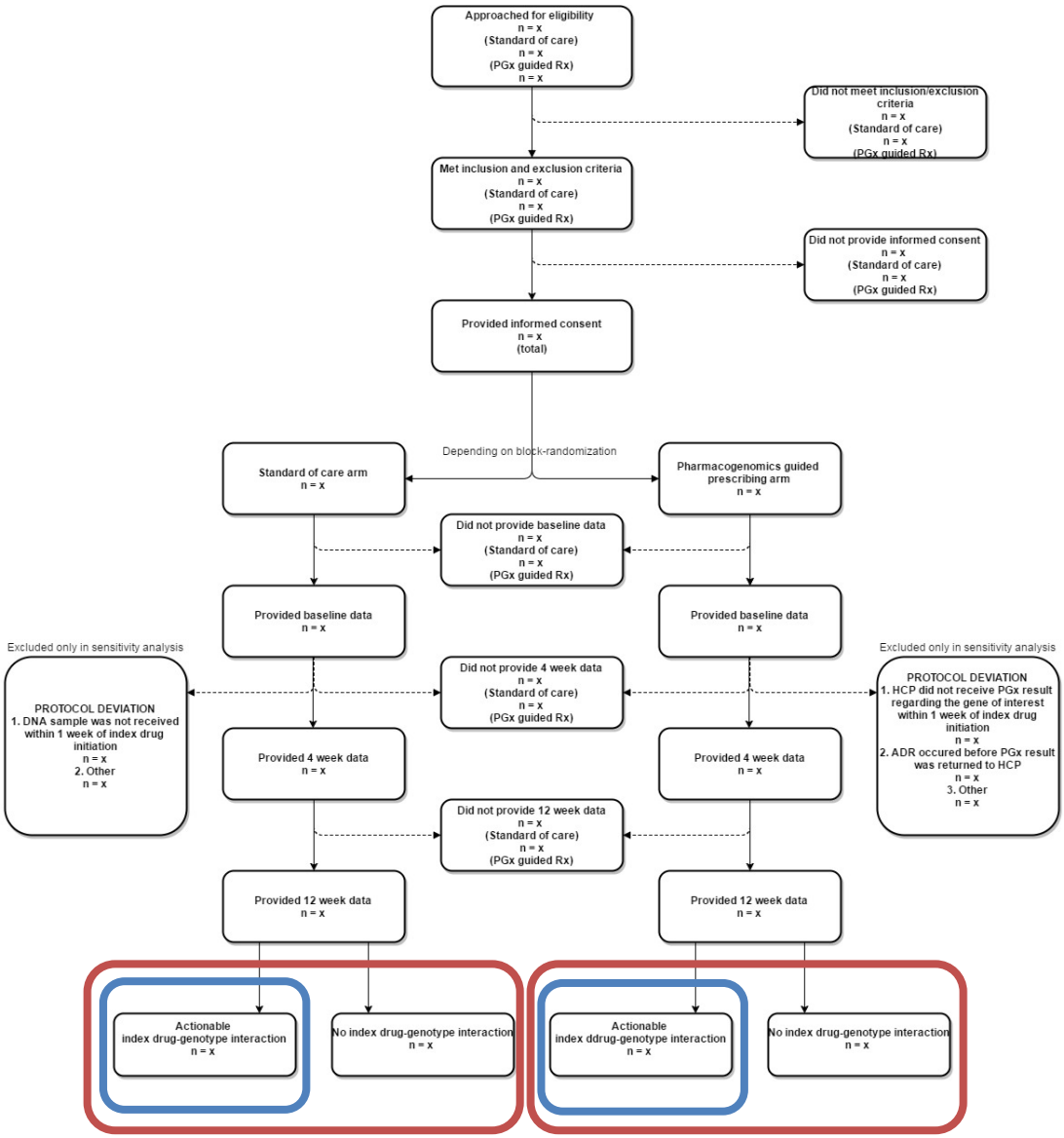

Figure 3 A schematic overview of the first and second test performed per country in the primary gatekeeping analysis

Per protocol analysis

In addition to the intention-to-treat analysis, a sensitivity analysis will be carried out following the same methodology, but exclude any patients in the study arm who reached the primary composite endpoint before their prescription was altered as per the DPWH guidelines and exclude any patients defined as having a major protocol deviations.

Table 4 Reporting the primary analysis:

	Frequency of ADRs contributing to the primary composite endpoint			**Frequency of ADRs contributing to the primary composite endpoint**		
Country	Actionable in standard of care arm (%)	Actionable in PGx guided prescribing arm (%)	OR [95% CI]	Standard of care arm (%)	PGx guided prescribing arm (%)	OR [95% CI]
NL						
UK						
ESP						
SLO						
AUS						
GRE						
IT						
Pooled analysis						

Table 5 Descriptive overview of clinically relevant ADRs contributing to the primary endpoint:

Country	**Overview of ADR characteristics**	**Actionable in standard of care arm**	**Not actionable in standard of care arm**	**Actionable in PGx guided prescribing arm**	**Not actionable in PGx guided prescribing arm**
NL (N)	Severity 2 Severity 3 Severity 4 Severity 5 Causality Possible Causality Probable Causality Definite MEDra term: -list Related index drug: -list				
UK (N)	Severity 2 Severity 3 Severity 4 Severity 5 Causality Possible Causality Probable Causality Definite MEDra term: -list Related index drug: -list				
ESP (N)	Severity 2 Severity 3 Severity 4 Severity 5 Causality Possible Causality Probable Causality Definite MEDra term:				

	-list Related index drug: -list
SLO (N)	Severity 2 Severity 3 Severity 4 Severity 5 Causality Possible Causality Probable Causality Definite MEDra term: -list Related index drug: -list
AUS (N)	Severity 2 Severity 3 Severity 4 Severity 5 Causality Possible Causality Probable Causality Definite MEDra term: -list Related index drug: -list
GRE (N)	Severity 2 Severity 3 Severity 4 Severity 5 Causality Possible Causality Probable Causality Definite MEDra term: -list Related index drug: -list
IT (N)	Severity 2 Severity 3 Severity 4 Severity 5 Causality Possible Causality Probable Causality Definite MEDra term: -list Related index drug: -list
TOTAL	Severity 2 Severity 3 Severity 4 Severity 5 Causality Possible Causality Probable Causality Definite MEDra term: -list Related index drug: -list

13. Analysis of secondary outcomes

The numbered analyses below correspond to the numbered secondary endpoints in chapter 5.2 Secondary outcomes.

1. The primary analysis will be repeated by treating NCI-CTCAE grade (2, 3, 4, or 5) as ordinal outcome. Logistic ordinal regression will be used in the analysis.
2. Occurrence of at least one ADR which contributes to the primary composite endpoint within the entire follow-up of the study.
    a. Statistical comparison between the two study arms will be performed using the same analysis as used in the primary analysis (gatekeeping analysis), except data originating from subsequent drugs will also be included in the data set.
3. Occurrence of at least one serious ADR within 12 weeks of follow-up.
    a. Statistical comparison between the two study arms will be performed using the same analysis as used in the primary analysis.
4. Number of self-reported ADEs through the LIM survey
    a. Statistical comparison between the two study arms will be performed using the same analysis as used in the primary analysis, except linear regression will be used instead of logistic regression.
5. Number of serious self-reported ADEs through the LIM survey
    a. Statistical comparison between the two study arms will be performed using the same analysis as used in the primary analysis, except linear regression will be used instead of logistic regression.
6. Number of dose adjustments made to the index drug
    a. Statistical comparison between the two study arms will be performed using the same analysis as used in the primary analysis, except linear regression will be used instead of logistic regression.
7. Drug cessation of index drug due to an ADR
    a. Statistical comparison between the two study arms will be performed using the same analysis as used in the primary analysis.
8. Drug cessations of index drug due to lack of efficacy
    a. Statistical comparison between the two study arms will be performed using the same analysis as used in the primary analysis.
9. Number of additional drugs that are prescribed during follow-up
    a. Statistical comparison between the two study arms will be performed using the same analysis as used in the primary analysis, except linear regression will be used instead of logistic regression.
10. Routine drug levels (only those that are collected routinely)
    a. Analysis will be performed per drug. Statistical comparison between the two arms will be performed using an unpaired t-test when normally distributed or a Mann-Whitney U test when not normally distributed.
11. Difference in patient-reported drug adherence score between baseline and 18 months.
    a. Analysis will be performed per drug. Statistical comparison between the two arms will be performed using an unpaired t-test when normally distributed or a Mann-Whitney U test when not normally distributed.

14. Analyses of missing data

14.1. Primary outcome

ADR data from patients who have failed to provide 4 week follow-up, but have provided 12 week follow-up will be used as is. Since ADRs which have occurred between 0 and 4 weeks will still be collected.

ADR data from patients who have provided 4 week follow-up, but have failed to provide 12 week follow-up will be imputed using multiple imputation taking into account covariates (center, age, number of drug allergies, number of co-medications and global health score).

Missing severity assessment

Severity will be assessed using the CTCAE. If this is missing the patient reported severity will be used as a severity score and will be calculated based using the definitions of the CTCAE.

Missing causality assessment

Causality will be assessed using the LCAT. If this is missing then local researchers will reassess the causality using data from the eCRF.

Missing drug-genotype association assessment

Since the drug-genotype association assessment will be performed by a blinded expert panel, in bulk, at the end of the study, we do not anticipate any missing data unless the DNA sample is missing or PGx panel results failed.

14.2. Secondary outcomes

When the incidence of the missing secondary endpoint data is less than 20% then the last observed value is carried forward.

When the incidence of the missing secondary endpoint is more than or equal to 20% then the missing data will be imputed using multiple imputation taking into account covariates (center, age, number of drug allergies, number of co-medications and global health score).

15. Complimentary statistical analysis plan: pre-emptive pharmacogenomics testing approach

All primary and secondary analyses (with the exception of secondary analysis 1) described in this statistical analysis plan are related to data collected regarding the index drug of inclusion only. The PREPARE study enables data collection not only for the index drug but also for (multiple) subsequent drugs. For the index drug, the PGx intervention includes a (maximum) seven day delay, where in the prescription of the index drug is not altered based on the patient's PGx results. This delay is due to logistical reasons. This delay could underestimate the true effect of pharmacogenomics guided prescribing, since patients start the index drug on an un-optimized dose or drug to bridge the seven day delay.

To assess the impact pharmacogenomics guided prescribing without this seven day delay (a truly "pre-emptive" approach) the analyses described above (with the exception of secondary analysis 1) will be re-run, in separate statistical analyses, using data collected regarding: only subsequent drug 1, only subsequent drug 2, only subsequent drug 3 etc.

16. Investigator blinding

To ensure the investigators are unable to influence the outcome of the trial the following steps will be undertaken:

1. The incidence of the primary outcome will only be determined once the expert panel has performed the drug-genotype assessments. To ensure the investigators will not be able to adjust the syntax according to this, the preliminary syntax for the primary analysis will be uploaded onto clinicaltrials.gov before the expert panel starts performing the drug-genotype assessments. We aim to upload the syntax before October 1st 2020.
2. The drug-genotype assessments will be merged with the final analysis dataset (expected November 2020).
3. The final primary analysis will be performed using the syntax uploaded onto clinicaltrials.gov (expected November 2020).

To ensure transparency and demonstrate investigator blinding, the table below will be filled in accordingly:

Task	**Aimed date**	**Actual date**
Date preliminary syntax to be uploaded onto clinicaltrials.gov	September 30th 2020	
Start date drug-genotype assessments	October 1st 2020	
End date drug-genotype assessments	October 31st 2020	
Perform final primary analysis	November 1st 2020	

17. References

1. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm
2. Gallagher RM, et al. Development and inter-rater reliability of the Liverpool adverse drug reaction causality assessment tool. PLoS One 2011;6(12):e28096.
3. Swen JJ. *et al.* Pharmacogenetics: from bench to byte--an update of guidelines. Clin Pharmacol Ther. 2011 May;89(5):662-73.

4. Swen JJ. *et al.* Translating pharmacogenomics: challenges on the road to the clinic. PLoS Med. 2007 Aug;4(8):e209.

18. Approval and agreement

This version of the SAP should be approved. SAP version 2.0 should be created and saved as a PDF after it has been reviewed by all members of the Executive Board and signed-off to ensure all are in agreement with the planned analysis and no further changes are foreseen.

SAP Version Number being approved: 2.0

Approved by: **Statistical Advisor** Name: Dr. Stefan Böhringer	Date: 4-8-2020

Approved by: **Chair Executive Board** Name: Prof. Dr. Henk-Jan Guchelaar	Date: 25/06/2020

Approved by: **Lead Component 1** Name: Dr. Matthias Samwald	Matthias Samwald Date: 24.6.2020

Approved by: **Lead Component 2** Name: Dr. Jesse Swen	Date: 25/6/2020

Approved by: **Lead Component 3** Name Prof. Dr. Matthias Schwab	29.06.2020 Date:

Approved by: **Lead Component 4** Name: Dr. Christina Mitropoulou	 Date: 29/06/2020

19. Appendix

Appendix A: CONSORT flow diagram regarding sample used in primary analysis